TA-65® reduces the telomere loss that accompanies normal aging

Dr. Jerry Shay
University of Texas Southwestern Medical Center, Department of Cell Biology, Dallas, TX, USA

Aim:
To compare the effects of oral intake of placebo and TA-65MD® (1000 Units) on telomere length in humans.

Background:
TA-65® is a plant-derived telomerase activator that has been shown to increase telomerase activity and lengthen telomeres in a variety of *in vitro* and *in vivo* model systems[1-4]. Recently, a randomized, double-blind, placebo-controlled clinical study demonstrated that oral intake of TA-65MD® has a beneficial impact on telomere length in humans[5]. A total of 117 subjects were recruited for that study; median telomere length and 20th percentiles of telomere length of the study subjects were determined using Q-FISH technology at Life Length (Spain). This method is based on a quantitative fluorescence in situ hybridization method modified for cells in interphase[6], and has been used by other research groups to measure telomere length[7]. Nevertheless, cognizant of the availability of many techniques to measure telomere length, we sought to measure telomere length using an alternative technique, TeSLA (Telomere Shortest Length Assay), to rigorously evaluate telomere length from a subset of samples from this large study.

Since age and gender have been shown to influence telomere length[8,9], we sought to minimize such confounding factors by analyzing age- and gender-matched samples. A previous preliminary assessment using TeSLA indicated that oral intake of TA-65MD® can decrease the percentage of short telomeres[10], essentially reaffirming the conclusions derived from Life Length's measurements. However, only four subjects' samples were tested in that pilot testing. Here, we expanded the pilot testing incorporating a larger sample number of age- and gender-matched controls to evaluate the mean telomere length and percentage of short telomere length (1.6 kb or less) using TeSLA, an assay developed in Dr. Shay's lab at the University of Texas Southwestern Medical Center[11].

Materials and Methods:
Mean telomere length and percentage of short telomeres were measured by TeSLA. A total of twelve samples from the placebo group (age range, 51 – 64 years; mean 57 ± 4 S.D.) and thirteen samples from the TA-65MD® (1000 Units) group (age range 50 – 72 years; mean 62 ± 6 S.D.) were analyzed at the baseline and at the end of the study (12 months).

Results:
In the placebo group, the mean telomere length decreased from 4.22 ± 0.42 kb at baseline to 3.92 ± 0.32 kb after 1 year, and the resulting loss of 300 bp is statistically significant (p=0.0002). In contrast, the change in mean telomere length (3.99 ± 0.59 kb at baseline to 3.95 ± 0.62 kb at 1 year) is not statistically significant (p=0.36) during the same time interval in the TA-65MD® group (Table 1, Figure 1). In order to see if the differences in telomere loss in these two groups over time were statistically significant, a multilevel statistical analysis was run. Based on this analysis, the change in telomere length of the TA-65MD® group compared to the placebo group over 12 months is statistically significant (p=0.0356) (Table 2). Similar observations were made in a previously published study[5]. Taken together, our results indicate that, while the mean telomere length decreased in the placebo group, it remained virtually unchanged after 1 year in the TA-65MD® group. Therefore, we conclude that oral intake of TA-65MD® might prevent the attrition of telomeres that normally occurs during aging.

Table 1: *Mean telomere length at baseline and after 1 year in age- and gender-matched subjects from placebo and TA-65MD®(1000 Units) groups*

	Telomere Length in kb (mean ± S.D.)			
	Baseline	***After 1 year***	***Change in mean TL†***	***p value****
Placebo (n= 12)	4.22 ± 0.42	3.92 ± 0.32	**-300 bp**	**0.0002**
TA-65MD® (n=13)	3.99 ± 0.59	3.95 ± 0.62	-40 bp	0.36

** Paired t-test was performed to derive p value; p value <0.05 is indicated by bold font. † Change in mean telomere length (bp) = mean TL after 1 year – mean TL at baseline.*

Stalling of Mean telomere length in the TA-65MD® (1000 Units) group

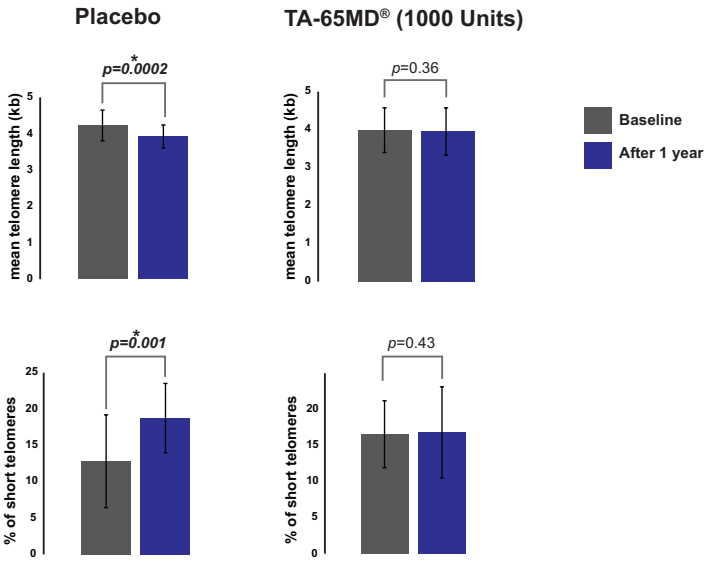

Figure 1: *Mean telomere length (upper panel) and percentage of short telomeres (lower panel) at baseline and after 1 year in age- and gender-matched subjects from placebo and TA-65MD® (1000 Units) groups; p values <0.05 are indicated in bold font with asterisk sign on top.*

Table 2: *Multilevel Model Analysis of Mean TL changes*

Effect	**Group**	**Time**	**Change in TL (kb)**	**S.E.**	***p* value**
Group effect	Placebo	Baseline		Reference	
	TA-65MD®	Baseline	-0.2388	0.2067	0.2599
Time effect	Placebo	Baseline		Reference	
		1 year	-0.3017	0.08551	0.0018
Group and Time effect	TA-65MD®	1 year	+0.2647	2.23	0.0356

Placebo and TA-65MD® (1000 Units) groups are compared at baseline and after 1 year for mean telomere length (TL). The data show change in TL in comparison with that of the reference group (placebo). SE, standard error.

Reduction of percentage of short telomeres in the TA-65MD® (1000 Units) group

Given the involvement of short telomeres in thwarting normal cellular function[12], we measured the percentage of telomeres shorter than 1.6 kb in both groups. As shown in Table 3, the percentage of short telomeres in the placebo group significantly increased from 12.81% at baseline to 18.75% after 1 year, and the resultant 5.94% increment is statistically significant (p=0.001). In contrast, the change in the percentage of short telomeres (16.54% at baseline to 16.80% at 1 year) is not statistically significant (p=0.43) during the same time interval in the TA-65MD® group. Similar to mean telomere lengths, we ran a multilevel analysis (Table 4). Based on this analysis, the difference in the percentage of short telomeres in the TA-65MD® group compared to the placebo group over 12 months is statistically significant (p=0.0108). Similar observations were made in a previously published study[5]. Taken together, our results indicate that, while the percentage of short telomeres increased in the placebo group, it remained virtually unchanged after 1 year in the TA-65MD® group. Therefore, we conclude that oral intake of TA-65MD® might prevent accumulation of short telomeres.

Table 3: *Percentage of short telomeres at baseline and after 1 year in age- and gender-matched subjects from placebo and TA-65MD® groups*

	Percentage of short telomeres (% ± S.D.)			
	Baseline	***After 1 year***	***Change in the % of short telomeres†***	***p value****
Placebo (n= 12)	12.81 ± 6.39	18.75 ± 4.77	+ 5.94%	**0.001**
TA-65MD® (n=13)	16.54 ± 4.64	16.80 ± 6.31	+ 0.26%	0.43

** Paired t-test was performed to derive p value; p value <0.05 is indicated by bold font. † Change in the % of short telomeres = % of short telomeres after 1 year – % of short telomeres at baseline*

Table 4: *Multilevel Model Analysis of % of short telomeres*

Effect	Group	Time	Change in % of short telomeres	S.E.	*p* value
Group effect	Placebo	Baseline		Reference	
	TA-65MD®	Baseline	3.7379%	2.2193	0.1056
Time effect	Placebo	Baseline		Reference	
		1 year	5.9400%	1.4787	0.0005
Group and Time effect	TA-65MD®	1 year	-5.6885%	2.0506	0.0108

Placebo and TA-65MD® (1000 Units) groups are compared at baseline and at the end of 12 months for % short telomeres. The data show change in comparison with that of the reference group (placebo). SE, standard error.

As the telomere length has been shown to vary between men and women[9], we analyzed telomere length in men and women separately. In the placebo group comprising women, the mean telomere length decreased from 4.15 kb at baseline to 3.82 kb after 1 year, and the resulting loss of 330 bp is statistically significant (p=0.003). Similarly, in men, the mean telomere length decreased from 4.30 kb at baseline to 4.03 kb after 1 year, and the resulting loss of 270 bp is statistically significant (p=0.023). Taken together, we conclude that the mean telomere length significantly decreases in both men and women cohorts of the placebo group.

In the TA-65MD® group, however, the changes in mean telomere length are not statistically significant in both men (p=0.47) and women (p=0.33) between baseline and after 1 year (Table 5, Figure 2). Therefore, we conclude that the mean telomere length remains virtually unchanged in both men and women in the TA-65MD® group after 1 year. Taken together, regardless of gender, the mean telomere length significantly decreased in the placebo group, while it remained virtually unchanged after 1 year in the TA-65MD® group. Therefore, we conclude that oral intake of TA-65MD® might prevent the attrition of telomeres that normally occurs during aging in both men and women.

Mean telomere length in men and women who took placebo or TA-65MD®(1000 Units)

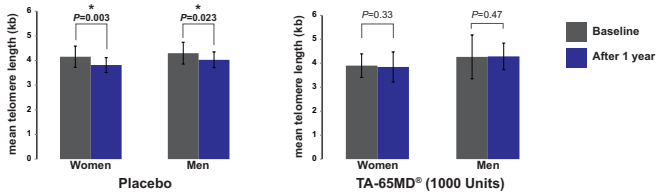

Figure 2: *Mean telomere length in kb at baseline and after 1 year in age-matched men and women from placebo and TA-65MD® (1000 Units) groups; p values <0.05 are indicated in bold font with asterisk sign on top.*

Table 5: *Mean telomere length at baseline and after 1 year in age-matched men and women from placebo and TA-65MD® (1000 Units) groups*

		Telomere Length in kb (mean ± S.D.)			
Group	***Gender***	***Baseline***	***After 1 year***	***Change in mean TL†***	***p value****
Placebo	Women	4.15 ± 0.43	3.82 ± 0.30	-330 bp	**0.003**
	Men	4.30 ± 0.44	4.03 ± 0.32	-270 bp	**0.023**
TA-65MD®	Women	3.90 ± 0.49	3.85 ± 0.63	-50 bp	0.33
	Men	4.27 ± 0.92	4.29 ± 0.56	+20 bp	0.47

** Paired t-test was performed to derive p value; p value <0.05 is indicated by bold font. † Change in mean telomere length (bp) = mean TL after 1 year – mean TL at baseline*

Finally, we analyzed the percentage of short telomeres in men and women in both groups. As shown in Table 6 and Figure 3, the percentage of short telomeres significantly increased in both men and women who were on placebo. In contrast, the changes in the percentage of short telomeres were not statistically significant in both men and women who were on TA-65MD®. We conclude that oral intake of TA-65MD® might prevent the accumulation of short telomeres.

Table 6: *Percentage of short telomeres at baseline and after 1 year in age-matched men and women from placebo and TA-65MD®(1000 Units) groups*

		% of short telomeres			
Group	***Gender***	***Baseline***	***After 1 year***	***Change in % of short telomeres†***	***p value****
Placebo	Women	12.61 ± 8.87	18.53 ± 5.76	+5.92 %	**0.038**
	Men	13.01 ± 3.33	18.97 ± 4.11	+5.96 %	**0.009**
TA-65MD®	Women	16.55 ± 4.33	17.28 ± 6.65	+0.73 %	0.35
	Men	16.52 ± 6.69	15.19 ± 5.89	-1.33 %	0.17

** Paired t-test was performed to derive p value; p value <0.05 is indicated by bold font. † Change in the % of short telomeres = % of short telomeres after 1 year – % of short telomeres at baseline*

Percentage of short telomeres in men and women who took placebo or TA-65MD® (1000 Units)

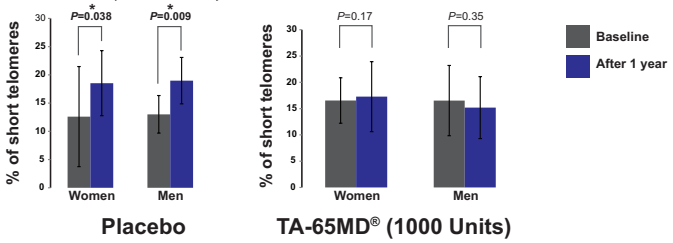

Figure 3: *Percentage of short telomeres at baseline and after 1 year in age-matched men and women from placebo and TA-65MD® (1000 Units) groups. P values <0.05 are indicated in bold font with asterisk sign on top.*

Conclusion:

In the placebo group, the mean telomere length significantly shortened and the percent of the short telomeres significantly increased over one year. In the TA-65MD® (1000 Units) group, the mean telomere length and the percent of the short telomeres did not change significantly over one year. These results strongly support that TA-65MD® reduces the telomere losses that occur with normal aging.

References:

1. Bernardes de Jesus B, Schneeberger K, Vera E, Tejera A, Harley CB, Blasco MA. The telomerase activator TA-65 elongates short telomeres and increases health span of adult/old mice without increasing cancer incidence. *Aging cell.* 2011;10(4):604-621.

2. Fauce SR, Jamieson BD, Chin AC, et al. Telomerase-based pharmacologic enhancement of antiviral function of human CD^{8+} T lymphocytes. *Journal of immunology*. 2008;181(10):7400-7406.

3. Molgora B, Bateman R, Sweeney G, et al. Functional assessment of pharmacological telomerase activators in human T cells. *Cells*. 2013;2(1):57-66.

4. Reichert S, Bize P, Arrive M, Zahn S, Massemin S, Criscuolo F. Experimental increase in telomere length leads to faster feather regeneration. *Experimental gerontology*. 2014;52:36-38.

5. Salvador L, Singaravelu G, Harley CB, Flom PL, Suram A, Raffaele JM. A Natural Product Telomerase Activator Lengthens Telomeres in Humans: A randomized, double blind and placebo controlled study. *Rejuvenation research*. 2016.

6. Canela A, Vera E, Klatt P, Blasco MA. High-throughput telomere length quantification by FISH and its application to human population studies. *Proc Natl Acad Sci U S A*. 2007;104(13):5300-5305.

7. de Rooij SR, van Pelt AM, Ozanne SE, et al. Prenatal undernutrition and leukocyte telomere length in late adulthood: the Dutch famine birth cohort study. *Am J Clin Nutr.* 2015;102(3):655-660.

8. Benetos A, Okuda K, Lajemi M, et al. Telomere length as an indicator of biological aging: the gender effect and relation with pulse pressure and pulse wave velocity. *Hypertension.* 2001;37(2 Pt 2):381-385.

9. Berglund K, Reynolds CA, Ploner A, et al. Longitudinal decline of leukocyte telomere length in old age and the association with sex and genetic risk. *Aging.* 2016;8(7):1398-1415.

10. Shay J. Randomized, double-blind, placebo-controlled study suggests oral intake of TA-65® can reduce the percentage of short telomeres in humans. *TA Sciences' Internal Document.* 2015;2015(004):2.

11. Shay J. Recent Advances in Telomeres and Telomerase: TeSLA, TPE-OLD and 6-thio-2'-deoxyguanosine. Telomere & Telomerase Meeting Suzhou, China 2016;*https://www.csh-asia.org/2016meetings/TELO.html.*

12. Hemann MT, Strong MA, Hao LY, Greider CW. The shortest telomere, not average telomere length, is critical for cell viability and chromosome stability. *Cell.* 2001;107(1):67-77.